Determination of growth in bony fishes from otolith microstructure

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Determination of growth in bony fishes from otolith microstructure

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PREPARATION OF THIS PAPER

This paper is a product of the André Mayer research fellowship "Ageing Fish Through Otolith Microstructure", awarded to the author from February 1987 to May 1988. In the course of the fellowship, in addition to other original research work, Dr Beatriz Morales-Nin prepared a first draft of this manual, which was later finalized for publication by FAO. The subject, though of interest to all scientists working on fish growth and ageing, is thought to be especially useful to those working with tropical species, where the traditional methods based on the interpretation of annual growth rings are particularly hard to apply.

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ABSTRACT

Fish ageing methods based on growth increments in otoliths are described in detail. A short introduction describes otolith extraction, preparation and viewing techniques, emphasizing the study of microscopic daily growth increments. Light and scanning electron microscope viewing methods and criteria for interpretation are described. The need to test the findings is explained and age validation techniques described.

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1 INTRODUCTION

The determination of fish age and growth is fundamental in fisheries biology and management. Such age-determined parameters as mortality and growth underlie the population dynamics models used in fishery analyses. Age studies can furnish other basic data such as stock age structure, age at first maturity, spawning frequency, individual and stock responses to changes in the habitat, recruitment success, etc. Age and growth data also permit the determination of population changes due to fishing rates.

Age can be determined by one or more of the following methods.

- Anatomical method: counting the regular growth marks formed in hard tissues such as scales, otoliths, vertebrae, spines and tail bones.

- Length-frequency analysis: monitoring the progression through time of the identifiable modes in size classes.

- Direct estimate: through direct measurements of growth rate of specific specimens extrapolated to the stock as a whole. Marking and subsequent recapture of fish, or monitoring the growth of captive fish of known age are two direct estimation methods.

Ageing tropical fishes was until recently assumed to be virtually impossible due to continuous spawning and the absence of growth cycles (Mohr, 1921), making the application of the anatomical method and of length-frequency analyses difficult if not impossible. It has been demonstrated, however, that though tropical fish have a longer spawning period than temperate fish (Lowe-McConnell, 1987), recruitment is limited to one or two seasons of the year. This limitation may arise out of spawning fluctuations or out of the juvenile and larval mortality which governs and limits recruitment to specific periods (Bakun et al., 1982; Victor, 1982; Robertson et al., 1988).

The deposition of annual growth rings (annulae) in the calcified tissues of bony fishes is at least partly caused by seasonal changes in the environment. These periodic changes (temperature cycles, available food) are less regular and less severe in tropical than in temperate zones. Several authors do, however, mention the presence of annual growth rings in tropical fish otoliths (Poinsard and Troadec, 1966; Quasim, 1973; Manooch III, 1987). The causes of this cyclical annual

growth are unclear: some authors link them to spawning periods and others to water temperature changes. As annual growth rings are present in immature fish, ring formation probably follows an internal rate of growth synchronized to seasonal environmental variations.

A new application of otolith growth structure analysis was developed by Pannella (1971; 1974; 1980), who showed that the concentric shells (microscopic lamellae) (Hickling, 1931) were formed daily. Daily growth increments offer a very promising field of study with many applications (Campana and Neilson, 1985).

These anatomical methods make it relatively easy to determine age and growth (Bagenal and Tesch, 1978; Casselman, 1983; Beamish and McFarlane, 1987). Nonetheless, annulae and growth studies cannot assume specific periodicity in growth marks, and so this must be determined for each age class of the stock studied (Beamish and McFarlane, 1983).

The method of separation of the modal classes (considered to correspond to distinct age classes) of the length-frequency distribution was the first to be applied to the determination of growth (Petersen, 1891). The early tropical fish growth studies used length-frequency analyses. However, the superimposition of successive modal classes and the difficulty of collecting representative, non-selective population samples are a frequent source of problems in the application of this method (Mathews, 1974; Morgan, 1983, 1985; Morales-Nin, 1989).

The method of estimating growth directly by marking and subsequent capture has rarely been used for tropical species. Diseases due to handling or marking the fish, as well as erroneous measurements, can affect the estimate. Additionally, many specimens must be marked to ensure a sufficient number of recaptures for growth estimates. In practice, marking and recapture experiments are limited to hardy species which can survive the stress of handling out of the water and also be recaptured in sufficient quantities (Munro, 1982).

The right method to apply in each case will depend on the available data and the characteristics of the population under study, plus technical and cost factors (Mathews, 1987; Gulland, 1987). Additional time and experienced, expert staff will be needed for the interpretation of the otoliths (Williams and Bedford, 1974), whereas length analyses are based on rather easily obtained data which can be quickly processed. The evaluation of the relative cost of each method should, however, bear in mind the degree of precision of the findings (Gulland, 1987).

The relative uncertainty inherent in all growth determination methods suggests the use of two independent techniques to confirm the findings. The use of length-frequency analyses and the simultaneous interpretation of growth marks probably offers the best results. The international ICLARM/KSIR meeting on the theory and application of stock assessment methods based on length-frequency analyses, which was held in 1985 (Pauly, 1987), concluded that length-frequency analyses methods are made much more precise by the inclusion of information on growth obtained through the use of an independent method, usually based on otolith reading (Morgan, 1987).

This paper describes a growth determination method based on otolith microstructure analysis. Growth determination by length-frequency analysis has been described in other FAO publications (Sparre et al., 1989) and by other authors (Pauly, 1982; Morgan and Pauly, 1987; Csirke et al., 1987).

2 OTOLITH MICROSTRUCTURE

Bony fish otoliths are complex polycrystalline bodies which act as organs of balance in the inner ear (Carlstrom, 1963; Gauldie, 1988). The otoliths are primarily composed of crystallized calcium carbonate in the form of aragonite and of a fibrous, collagen-like protein: otoline (Degens et al., 1969; Morales-Nin, 1986 a; 1986 b). Partly or wholly abnormal otoliths made up of calcite are relatively common (Morales-Nin, 1985 a). These crystalline otoliths are transparent and lack clearly defined growth marks.

The otolith grows by the surface deposition of materials, a cyclical process dependent on internal calcium metabolism rates (Simkiss, 1974) and on amino-acid synthesis. The result is the formation of daily growth increments in the otolith, made up of a continuous or incremental unit, and a check unit (Pannella, 1971; 1974; Dunkelberguer et al., 1980). The incremental zone is made up of needle-like aragonite microcrystals surrounded by the organic matrix and laid down across the surface of the otolith. The check zone or unit is mainly made up of concentric shells of organic matter (Mugiya et al., 1981; Watabe et al., 1982; Morales-Nin, 1986 b).

The thickness of the increments and density of the microcrystals depends on the stage of growth (Irie, 1960). In active periods of growth, for example, the increments are thick with well-developed check units and in slow periods the increments are finer and the microcrystals more compact and continuous. Often,

there are two or more sub-increments, probably caused by migrations, feeding rates (Pannella, 1974; 1980) and temperature changes (Brothers, 1978; Pannella, 1980; Campana, 1983; Campana and Neilson, 1982; Geffen, 1982, 1983), etc.

As bodily growth and otolith growth are closely linked, the increment thickness will reflect the rate of growth, recording periods of environmental and physiological stress and growth fluctuations caused by age-linked metabolic slowdown (Gutiérrez and Morales-Nin, 1986). Bodily growth and otolith growth do in some cases, however, appear to occur independently (Wright et al., 1990).

The daily deposition of increments depends on circadian endocrine rhythms which are synchronized at an early age with photo-periodicity or other external daily factors (Tanaka et al., 1981; Radtke and Dean, 1982; Campana and Nielson, 1985). The synchronizing stimulus must either not vary in periodicity by more than 2-4 hours from the 24-hour cycle, or else must consist of harmonious multiple 24-hour cycles. Only an environmental factor can act as synchronizer, although other factors may mask or reinforce the endogenous rhythm.

The daily deposition of increments should, at least in theory, allow an extremely precise determination of age. Many authors have used these increases to determine the age of larvae and juveniles (see references in Ré, 1983; Campana and Nielson, 1985; Palomera et al., 1988; Bergstad, 1984; inter al.) and in some studies on adult fish (Darayatne and Gjosaeter, 1986; Ralston and Miyamoto, 1981; Randall, 1961; Morales-Nin and Ralston, 1990; Strushaker and Uchiyama, 1976; Taubert and Colbe, 1977; Uchiyama et al., 1986; Uchiyama and Struchaker, 1981). However, after the first year of life the thinness of the increments and otolith morphology can make interpretation difficult (Morales-Nin, 1988).

The daily periodicity of increments has not been determined in some cases, and the authors have simply assumed that it does occur, daily rhythms being common in marine organisms. This can induce error, and although determining the increment deposition rate at all ages is more work, it is essential if the results are to be considered valid.

In addition to age determination, increments have been used to validate annulae periodicity (Pannella, 1980; Victor and Brothers, 1982), to determine changes in growth (Gutiérrez and Morales-Nin, 1986), to detect life transitions (Radtke, 1984), to estimate recruitment and mortality (Methot, 1981, 1983; Robertson et al., 1988; Thomas, 1983) and in taxonomic studies.

3 OTOLITH REMOVAL AND STORAGE

3.1 Otolith selection and removal

The otoliths must be removed as soon as the fish dies, barring which the fish must be frozen or duly fixed to avoid the loss of the growth structures present in the otoliths. Larvae and juveniles must be handled with great care, because the area to otolith volume ratio makes them much more susceptible to deterioration.

There is a certain amount of body loss in fish storage, which is of particular importance for larvae and juveniles (Radtke, 1989; Kruse and Dalley, 1990). The corrective factors should be calculated by measuring and weighing a series of specimens, before and after preservation, covering the size range to be studied. Shrinkage is proportionate to fish size, and depends on storage time and preservatives (Kruse and Dalley, 1990). The period of time between death and fixing must also be reckoned for juveniles and larvae (Theilacker, 1980).

Being calcified, otoliths are broken down by acid fixatives such as formalin. The pH of buffered formalin can vary over time and it should therefore be used only for short periods. The fixative which produces the best results is ethanol, which should be used in a concentrated, 85 percent solution to compensate for dilution by the passage of bodily fluids into the storage medium. The alcohol should be changed from time to time for better storage. The addition of marble chips to the fixative will stabilize pH and improve otolith storage (Brothers, 1987).

Freezing is a good storage method where there is no risk of partial thawing due to temperature changes, which would degrade otolith quality.

In beginning the study of a species, the in situ position and orientation of the otoliths should be noted (asteriscus, lapillus, sagitta), so as to determine their relative morphology and orientation by their position in the saccule (Fig. 1). The size of the otolith may change throughout the fish's life, and so despite the fact that the sagittal otolith is the largest in many species, size should not be the sole means of differentiating the otolith.

The sagittal otolith is the one most commonly used, although some studies have employed the lapilli, which is smaller and requires less preparation (Brothers, 1987). In ageing, the same type of otolith must always be used as increment growth is not simultaneous in the three pairs of otoliths.

Both left and right otoliths of each fish should be collected and kept separate until their morphology can be differentiated (Hecht, 1978). The growth rings are usually identical in both otoliths: if one has been damaged during handling, or is crystalline, the other can be used.

To remove otoliths the cranium must be sectioned to reach the chambers of the inner ear. Cranial shape is filogenetic and therefore the removal technique must be modified to suit the species studied (Holden and Raitt, 1975). In round fish a transversal cut is usually made in the head a little behind the eyes. The cut must be deep enough to open the skull without damaging the otoliths. When the saccule is exposed the otoliths are withdrawn carefully with forceps so as not to break them. With small otoliths, it is better to remove the semicircular canals and separate the otoliths under a binocular microscope. Any adhering tissue can be removed from the otoliths by rubbing them gently between the fingers or with tweezers under a magnifying glass. Immersion in a five percent sodium hypochlorite solution facilitates the cleaning operation.

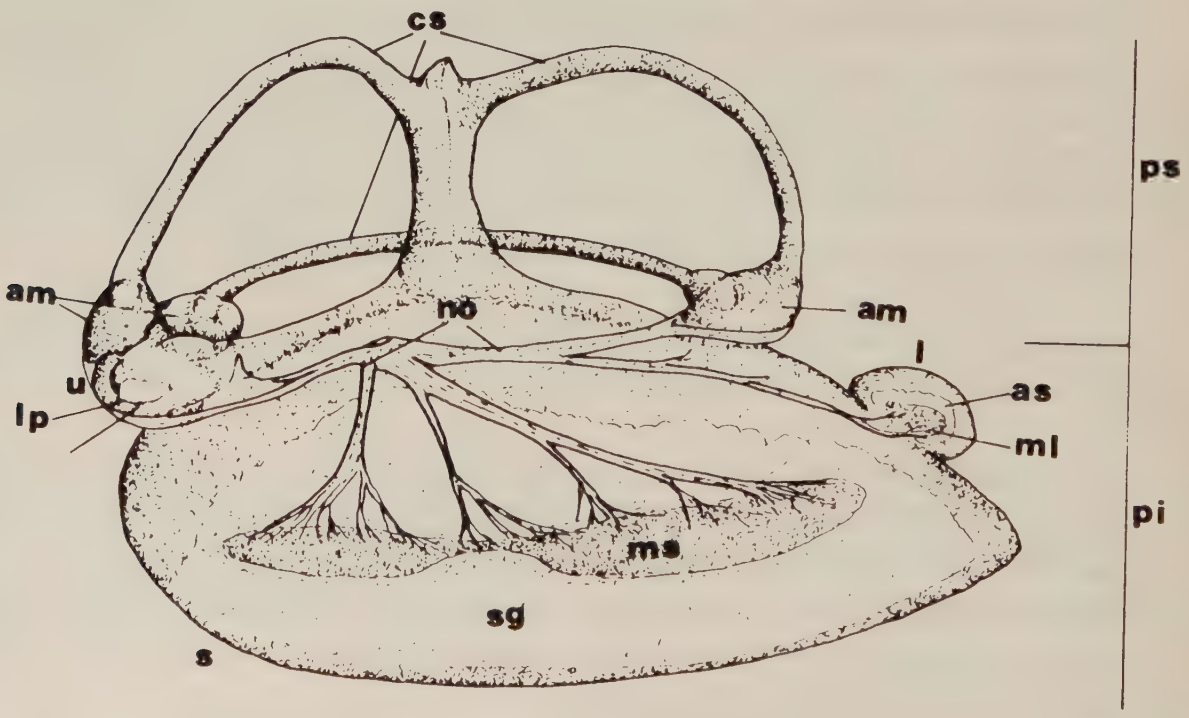

Fig. 1. Transverse section of right inner ear. am: ampulla, as: asterion, cs: semicircular canals, l: lagena, lp: lapillus, ml: macula lagenae, ms: macula sacculi, mu: macula utriculi, no: auditory nerve, pi: pars inferior, ps: pars superior, s: saccula, sg: sagitta, u: utricle.

3.2 Otolith storage and preservation

The otoliths must be stored in such a way as to ensure they occupy the minimum space, save money, and are well-preserved and easy to identify. As otoliths are acellular bodies with a small proportion of organic matter, the risk of decomposition is minimal. Growth structures are nonetheless more easily visible in newly collected otoliths. They should therefore be read as quickly as possible.

Otoliths can be stored in test-tubes or vials, dry or with a clarifying liquid when they are to be read immediately. Another method in use is to stick them with transparent nail varnish to a slide or with two-sided sticky tape to a sheet of acetate. The more sturdy otoliths are usually stored dry in properly labelled envelopes.

3.3 Removal and storage of larval otoliths

The small and transparent larvae can be mounted in a clear medium for direct viewing of the otolith interior. The slide cover is pressed gently to split the cranium and expose the otoliths, freeing them from the surrounding tissue.

It is relatively easy to remove larval otoliths of larger size when the larvae are transparent. Read under a lens with polarized light, the otoliths appear as points of light against a dark background. The larvae are fixed on a slide with a drop of water and the otoliths removed with a fine needle.

Leave the otoliths to dry for a few moments after removal and place them distal side down on the slide, covered with some neutral mounting medium like Euparal, Flo-texx, Depex, Permounta[1] to brighten the structure, and cover with a slide cover. The mounting medium shrinks as it dries and slide cover pressure can break the otoliths. To avoid this, place a piece of fishing line or other neutral material on both sides of the otolith in order to maintain a constant distance between the slide and the cover. The result should not be too thick as the microscope's working distance is small at the blow-up needed to read daily increments. When otoliths are mounted in Flo-texx-type media, which stiffen as they dry, the slide cover can be eliminated.

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1 The commercial brands mentioned in this document are indicative and in no way imply recommendation by FAO.

Mineral oil and immersion oil are mounting mediums which do not solidify and which allow later handling of the otoliths. The otoliths may, however, become unreadable after some time in such media (Brothers, 1987). The edge of the slide cover should be sealed with nail varnish or glue and the slides stored horizontally in a dust cover.

Canadabalsam is acidic, or can become so with time, and should therefore not be used to mount the delicate larval otoliths.

4 OTOLITH PREPARATION

The techniques to apply will depend on research needs, the life stage of the fish, otolith size and shape, and reading techniques. The increments are read with the light microscope or with the scanning electron microscope, both requiring special techniques.

Under normal viewing conditions and sample quality, the light microscope has a very short working distance and a resolution of 1 μm. Except for larval otoliths, otoliths are too thick and must therefore be sectioned for transparent reading.

The scanning electron microscope has very high resolution (a few Amstrongs depending on sample quality) but very little penetration power. Only the surface topography of the specimen is accessible for reading, and the otoliths must therefore be sectioned to read the internal structure. The chemical differences between the two units of the increments are what signals their presence on the surface of the otolith section (Fig. 2).

The scanning electron microscope should be used in otolith structural studies, in the verification of structures first read with the light microscope, and in species where the growth increments are too thin to be read under the light microscope.

4.1 Morphometric measurements

The otoliths must be weighed and measured before preparation, in order to determine the fish size/otolith measurement ratio. First determine otolith orientation using the standard terminology (see Glossary) to describe the exact location of the measurements.

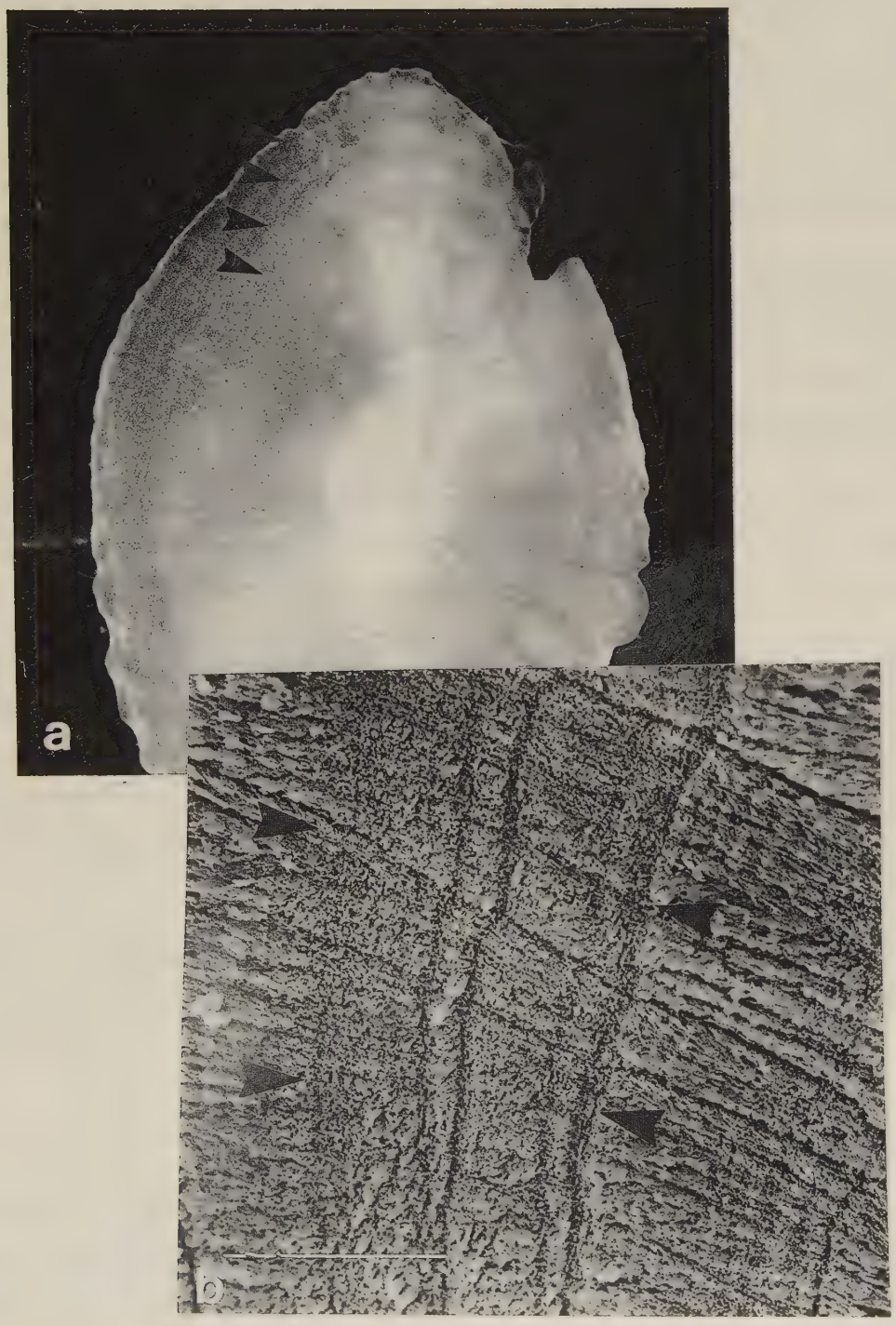

Fig. 2. Sagitta of Lutjanus kasmira showing the slow growth seasonal rings. a: read with binocular microscope and, b: read with scanning electron microscope (scale 10 μm).

The most frequent measurements are length (from rostrum to postrostrum) and maximum width, perpendicular to otolith length. When the central focus of the otolith is visible, other measurements are possible, such as radius (from the focus to the postrostrum) distance to the antirostrum, etc. Do not use broken or altered otoliths for morphometry.

Very small otoliths must be measured under the microscope using millimetre grids. Larger ones (> 1 cm) can be measured with calipers. All otoliths must be measured without changing their axial inclination: the small, fragile, edge markings which may be broken should not be counted.

Image analysis is a technique which provides objective data on otolith dimensions, allowing length, width and area to be calculated (Lombarte, 1990). The image is acquired through a high resolution television camera coupled to a computer, and transformed and analysed through image analysis. The stages of the process are:

The real image, caught by the camera, is transformed into an analog image which is digitalized by an analog transformer and real-time image processor. The digital image is composed of pixels: pixel size and light level determine image quality.

The real size of the digitalized sagitta is determined by calibration and calculation of the pixel equivalent in mm. Lastly, image analysis produces the biometric measurements of area, perimeter, length and height. Using these data as a basis, biodimensional morphometric measurements or the implied functions can be plotted to determine growth (Berman et al., 1984), differentiate populations or determine otolith biometry (Lombarte, 1990).

To avoid possible errors in weight caused by changes in moisture content (Pawson, 1990), the otoliths should be dried in a moderate oven (80°) until the weight remains constant. The otoliths are then kept in a dryer until it is time to weigh them.

4.2 Sectioning

Cross-sections are necessary to study increment sequence in the otolith. Sections across the various planes of the otolith (Fig. 3) can be cut or polished. All sections must be taken across the nucleus to avoid missing part of the increment sequence.

The orientation of the section is important as many otoliths grow asymmetrically. In sagitta with preferential growth in the internal or sulcal phase, transverse and diagonal cross-sections are recommended. In otoliths with preferential longitudinal

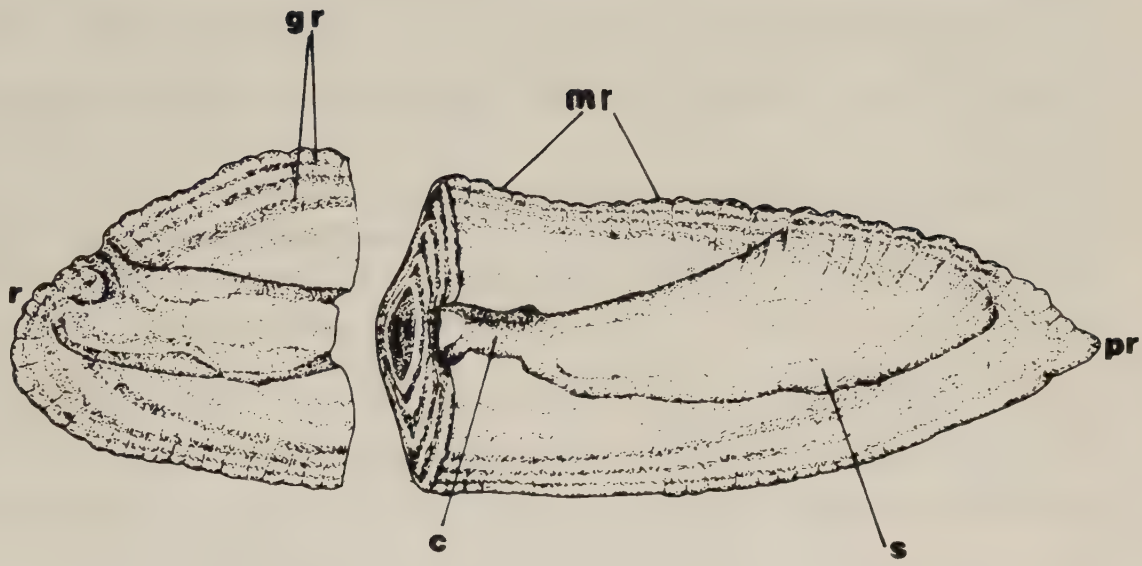

Fig. 3. Diagram showing the transverse section of the sagitta and its principal parts. r: rostrum, s: sulcus, c: collum, n: nucleus, pr: postrostrum, mr: dorsal crests, gr: annulae.

growth, the transverse sections may contain many growth checks, and therefore the frontal sections are to be used. In beginning the study of a species, otolith sections representative of the size range must be prepared, and the most suitable section plane determined.

The otoliths are cut with precision saws (Isomet type) with fine diamond blades. The otoliths should be enclosed in a plastic resin in order to obtain exact sections, so that the blocks can be easily positioned in the saw (Bedford, 1977; Rauck, 1975).

The otoliths are embedded in moulds (ice cube trays, semi-rigid plastic or metal, etc.). The moulds should be sprayed lightly with a silicone spray to facilitate subsequent extraction of the block. Pour a small amount of plastic into the mould and wait until the resin is semi-hardened. The otoliths are placed on the resin, lined up so as to facilitate subsequent cutting along the plane selected. Lastly, the moulds are filled with resin, carefully ensuring that no air bubbles remain. Each otolith must have enough resin around it to avoid it breaking when cut. The moulds are placed in a dust-free place and left to harden for 24 hours. If the blocks are still rather soft when extracted, they may be hardened in a moderate oven.

First eliminate all irregularities from each block, then determine the position of the nucleus, marking this on the block with a fine-point marking pen or diamond point. Mark the best section plane on the surface of the block with a ruler, leaving a margin for the thickness of the cutting knife. Then place the block on the saw arm so that the edge of the blade is lined up with the line made on the surface. The section is sawed at low rpm to avoid breaking the otolith and so that the heat from the friction will not melt the resin covering the section, which would make a subsequent reading difficult.

To prepare a fine section bring the blade forward (forward distance = desired thickness of section + blade thickness) and proceed to the second cut. When two diamond blades separated by a metallic spacer of the proper thickness are used, the section is obtained in a single operation. The section is glued to a slide with the face nearest the nucleus downwards and then ground to the right thickness for reading the increments.

To obtain transverse sections of large otoliths, place them on a semi-hard surface and break them across the nucleus using a knife. With a little practice you can control the blade pressure so as to obtain precision sections. This kind of section is useful for SEM readings and for studying the annulae.

Preparing sections for polishing is a two-stage process. First the otolith is ground down to the desired plane and polished to eliminate any surface irregularities. This is a painstaking process, the grinding plane must be constantly checked to avoid overshooting the nucleus or wearing away the otolith edges.

Small otoliths can be embedded in plastic with the polishing face up. The resulting block is easy to handle and will keep the otolith from breaking while handled. The otoliths can also be stuck on to a slide or fingertip with two-sided tape. Both faces of the otolith can be polished if necessary.

Small otoliths with some opaque zones can be etched with a weak acid solution rather than polished. The process of etching, washing and drying is repeated under the dissecting microscope until the desired otolith thickness is obtained. The acid (HC1 0.1 N) is very carefully applied with a fine-tipped paintbrush. The otolith edge can be protected with a bit of nail varnish, which is then removed with acetone. This method is valid only for the light microscope, as the resulting surface will be too irregular for SEM reading.

4.3 Preparing otoliths for light microscopy

The short working distance of the light microscope and the blow-up necessary to read growth increments (400-1 000 X), implies a very thin otolith section. Except for the small larval otoliths, which can be read directly (Fig. 4), all otoliths must be finely sectioned as described above.

The section is washed in water and dried under a gentle heat source such as a lamp or moderate oven (60°C) and mounted on a slide. Section slide mounting is identical to larval otolith mounting. The clarity of the growth increments increases after some time (2 to 3 weeks) in the mounting medium.

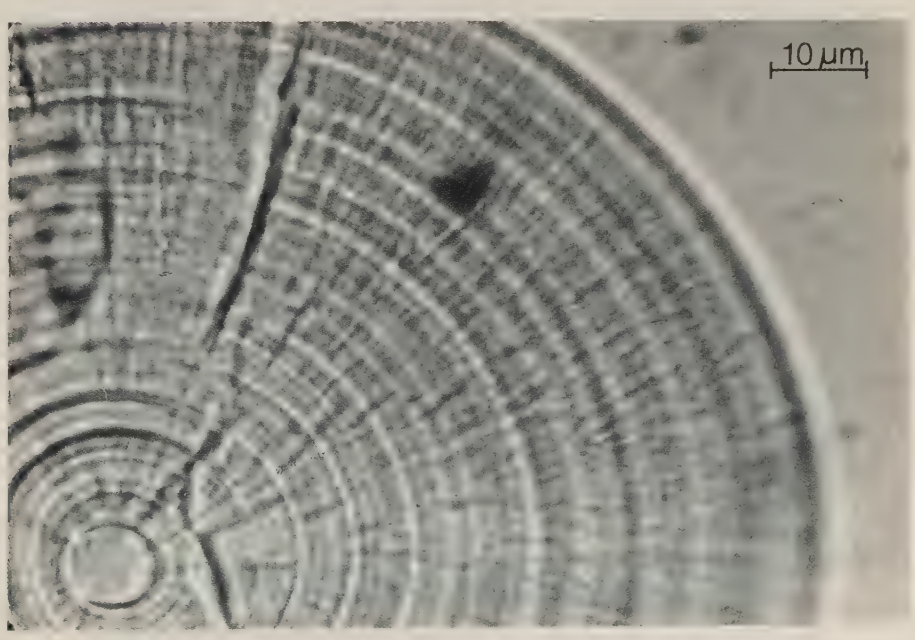

Fig. 4. Daily growth increments in an Engraulis encrasicholus larval otolith read under the optical microscope.

The otolith edge needs to be embedded in enough mounting medium to avoid optical deformations. The mounting medium shrinks as it dries, and so new medium must be added with a capillary tube. Leave the section to dry at a slant to allow air bubbles to escape.

Mounting media can be dissolved, if necessary, with a proper solvent such as toluene (for Pro-texx and Euparal) or xylene (for Flo-texx) and the otolith repolished and remounted.

4.4 Preparing otoliths for reading with the scanning electron microscope

Sections across the nucleus are prepared for viewing the internal structure of the otolith with the SEM. Reading is unaffected by the thickness of the section.

The sections are stuck to the SEM slide and ground with a fine polishing paste (0,3 μm) until the sample surface is mirror-like. The preparation is washed with distilled water (cleaning may be facilitated by using ultrasound), and etched with a weak HCl 0.1 N solution or with a 0.2 M EDTA solution to highlight the increment zones. Increment clarity and thickness depends on how and how long the sections are etched. The most appropriate interval must be determined for each specific case. First use a short interval (1 minute for EDTA, 30 seconds for HCl), if the prepared section is not sufficiently etched it can be re-polished and re-etched for a longer time.

Lastly, the otoliths are metallicized with palladium gold, the usual SEM technique, to increase conductivity.

4.5 Otolith replication

It may be impossible to prepare thin sections of large thick otoliths. For such otoliths, acetate replicas of the surface of a cross-section may be the only way to read increments.

Prepare the otolith as for SEM viewing: the otolith is etched in acid to bring out increment contrasts. The etched otolith surface is washed with acetone and carefully dried with a blower (e.g. an aquarium compressor). Spray the section lightly with a silicone spray, air-blowing off the excess silicone. The otolith section is positioned horizontally on a glass slide with plastiline (children's moulding clay).

The acetate paper must be thin - some 0.3 mm thick (Wild, 1982; Fagade pers. comm.) and kept perfectly dry. There are two replication methods:

1. Cut a thin strip of acetate paper with scissors, a bit bigger than the otolith, and immerse the edge of the acetate paper in acetone with forceps (do not wet the forceps). Immersion time in the acetone is calculated by dissolving a bit of acetate paper. Three-quarters of the time needed to dissolve the paper is the proper replication interval (Wild, 1982). Acetate paper dissolving time is particularly affected by temperature, which should therefore be read before starting the replication.

Gently press the surface of the otolith to be replicated onto the acetone-dipped acetate paper strip, using a spatula to eliminate air bubbles. After drying for 3-4 minutes, carefully separate the strip and the otolith to avoid fracturing the otolith.

2. Place a somewhat larger strip of acetate paper over the otolith, carefully depositing 1-2 drops of glacial acetate acid on the acetate paper. Be sure the acid

Fig. 5. Photomicrograph (scanning electron microscope) showing growth increments in a continuous unit (in relief) and a check. Scale 75 μm.

covers only the otolith area (Fagade pers. comm.). When the replica is dry, use forceps to remove it from the otolith.

While the replicas are drying, prepare slides by fixing a cover slip with a bit of transparent tape so that the slide and cover slip open like a book. Slip the replica into this and fix the cover slip with transparent tape, pressing gently.

The replicas can be read with the SEM like thin sections. Should the quality of the replica be poor because of errors in the preparation or poorly-done sections, the otolith can be re-polished and the process repeated.

5. AGEING

5.1 Viewing increments with the light microscope

The increments appear under the microscope as concentric rings which are alternately clear (continuous zones) and dark (discontinuous zones). Each pair forms a daily growth increment. Sub-zones often appear, i.e. small rings formed with variable periodicity and probably caused by the ingestion of food, environmental variations or stress (Pannella, 1980). Thick and well-marked sub-zones may make increment identification a complicated process. Generally speaking, sub-zones lose clarity or disappear when the focus is changed slightly. It is therefore recommended that you bring the preparation into a focus where all growth structures can be clearly read and then vary the focus slightly to differentiate sub-zone increments.

You need a good microscope and a strong source of light to read the increments: long focal length lenses will allow you to work with thicker preparations, up to 1 mm, making otolith preparation easier. Optical resolution is better when the condenser is positioned as close as possible to the preparation. Polarized light will strikingly increase the contrast between the increment zones.

The magnification for reading will depend on increment thickness, the general range being 400 - 1 000 x. Many species have very fine growth increments undetectable by the light microscope (Morales-Nin, 1989). These fine increments (Fig. 6) may appear as otolith zones without clearly defined growth structures (Mizendo, 1984) but under the SEM they can be seen to be made up of fine increments ($\pm$ 0.5 μm) (Morales-Nin and Ralston, 1990).

In beginning the study of a new preparation, it is advisable to locate the radius which most clearly shows the increments within a few degrees of magnification,

and then move the preparation until the nucleus remains in the centre of the visual field. Using the magnification required for reading, begin the count following the previously established radius. The structural characteristics of the otolith, checks, wider rings, etc. can act as markers, making it easier to localize up to where the count has been made.

It is very tedious and fatiguing and tiring to the eyes to count the increments in the otoliths of fish older than one year. Place a hair from a brush on the eye-piece to get a marker which will facilitate the count; a manual plankton recorder can be used to record the increments counted.

A number of semi-automatic systems have been developed to count increments using a modified image analysis apparatus. These systems can differentiate between the grey levels of the image and identify increments. A sub-routine of the system allows manual correction of identification errors and marking of any increments omitted.

Finally, calculate the total number of increments in the otolith and age the specimen.

Fig. 6. SEM photomicrograph showing fine increments ($< 1\ \mu m$) deposed during unfavourable growth periods of Sardinella longiceps (scale 15 μm). Observe the cyclical grouping patterns of the increments.

5.2 Viewing increments with the scanning electron microscope

Under the scanning electron microscope, increments appear as crests and sutures due to the different response of the zones to acid. The sub-zones are usually less marked due to the greater continuity of the microcrystals through them. SEM resolution is sufficient to read all increments, no matter how fine (Fig. 7).

The specific microscope characteristics will determine the working distance, the degree of inclination and the voltage to employ. Low voltages (15 KV) are recommended to avoid problems with specimen conductivity during the reading.

In measuring increments, the specimen surface must be positioned horizontally to the electronic face (a specimen positioned at a slant will produce distortions that will affect the measurements). Increments can be measured in photographs or on the microscope screen using acetate grid paper. The television signal of the SEM can be recorded with a video-recorder and later analysed on a TV screen.

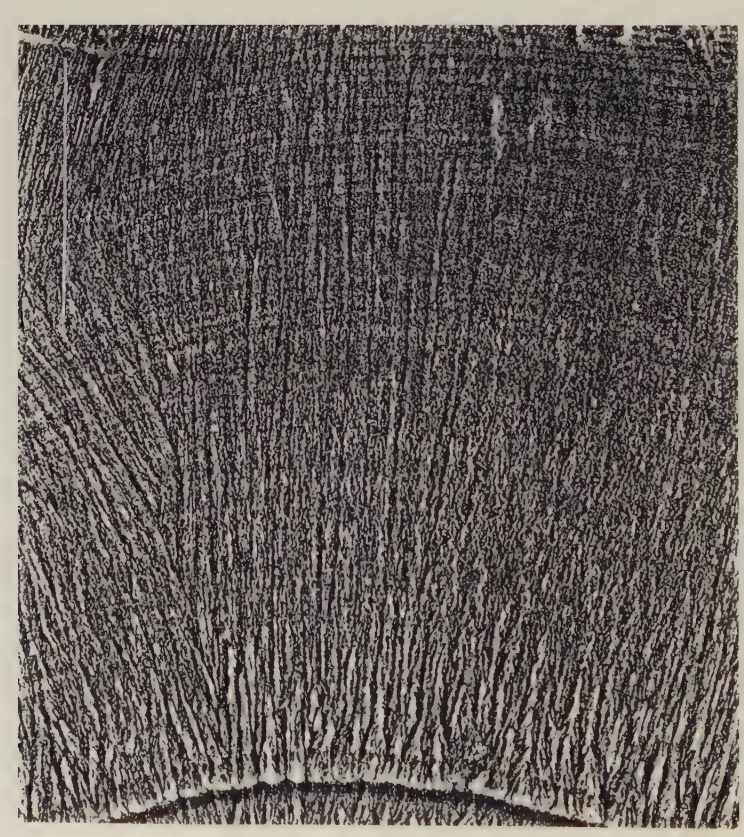

Fig. 7. Fine increment depositions on an Engraulis ringens otolith (scale 27 μm).

5.3 Ageing through analysis of increment microstructure

Age interpretation is a three-part process:

1. Identification of growth structures:

Several otoliths must be examined to define the increments whose periodicity will be considered daily. An increment can be counted when you can follow it around most of the otolith. When a discontinuous or check zone is found on the edge, the increment is to be considered as in the process of formation and is not counted. Increments on the edge of the otolith, however, are often masked by the optical distortion produced by the angle of refraction between the edge and the medium, which makes it difficult to count marginal increments.

It is advisable to begin to read otoliths of younger specimens, and, with practice, gradually progress to the otoliths of older fish.

2. Definition of interpretation criteria:

In starting to study a new species, the interpretation must be confirmed by several scientists who repeat the otolith reading and discuss the criteria for interpretation (Gjosaeter et al., 1983). A few otoliths covering the range of sizes to be studied are selected, interpreted and reinterpreted. This process allows the best area for otolith interpretation to be identified, as well as checking reader consistency and consensus. Variability among readers can be determined by some statistical test such as the t test. Unless agreement is good, the criteria for interpretation must be revised. Where interpretation and discussion do not produce reader consensus, this may be due to poor-quality preparations. The comparative analysis of the findings will show the type of error involved (Gjosaeter et al., 1983).

3. Age reading from a stock sample:

Once the otolith structures have been identified and the interpretation criteria defined, the individuals in the stock sample can begin to be aged. For maximum objectivity neither the size nor other data about the otolith must be known. Each otolith, identified by a reference number, must be read at least twice and only readings which coincide can be considered valid. Any discrepancies will depend on the age of the fish: the more increments there are the greater the discrepancies.

5.4 Ageing based on increment thickness

Ralston and Miyamoto (1981; 1983) and Ralston (1976; 1985) presented a method for ageing based on otolith increment thickness. Since otolith size depends on the age of the fish, which is closely correlated with length, increment thickness will reflect bodily growth (Campana and Nielson, 1985; Gutiérrez and Morales-Nin, 1986).

If otolith formation were constant, increment thickness would be uniform throughout the otolith. After determining the otolith growth rate, the age would be determined by dividing otolith size by the rate of growth. But as fish and otolith growth are both variable, this method can only be applied to small segments of the otolith where growth is considered uniform.

The segments usually used are 500 μm, and should not exceed 2% of the radius of the otolith (Ralston and Miyamoto, 1983).

The method involves the following steps:

1. Preparing the otolith for viewing with the light microscope.

2. Measuring the radius of maximum otolith growth starting from the focal point (R), where the otoliths are to be aged and measured.

3. Calculating the ratio between the otolith radius (R) and fish length (FL).

4. In each zone where the increments are visible: a) count the number of increments (t), b) measure the size of the zone, c) measure the distance (r) between the otolith nucleus and the mid-point of this zone.

5. The data for each otolith are summed up at 500 μm intervals from the radius r of each segment.

6. The linear relationship between the sets of data pairs dr/dt and r is calculated logarithmically for each otolith. The equation:

$$\ln(dr/dt) = c\text{-}br + a$$

where r is the length in millimetres, t days (number of increments), c,b, are regression constants, and a is a variable.

7. Fish age is determined by the integration of:

$$dt = c'e^{br}dr, \qquad c' = e^{-b}$$

where fish age (T) is: $T = c'/b(e^{br}r)$.

The Delta method (Seber, 1973) is used to find the integration.

The method has been critically evaluated through ageing by annual rings, Monte Carlo simulations, length frequency analyses and studies of spawning periods related to birthday determined by back calculation (Ralston and Williams, 1989).

Counting otolith increments in adult fish is extremely tedious and difficult and so this method is particularly useful for a semi-automatic application using an image analyser recently developed by Ralston. The method's major limitation is that in some species increment depositions during periods of slow growth are extremely fine (Fig. 7) and are therefore undetectable by light microscope. The zones with this type of increment will appear to lack clear growth structures and will not be included in the age calculations. Age will be underestimated if only the thicker increments are used, and this will give exaggeratedly high growth rates (Morales-Nin, 1989; Morales-Nin and Ralston, 1990).

5.5 Ageing by reading annulae

Many authors have reported the presence of seasonal growth rings (annulae) in tropical fish otoliths (Brothers, 1979; 1982; Sainbury and Whitelaw, 1984; Samuel et al., 1985) although ring deposition in some species is irregular (Mathews, 1974). Once the annual ring periodicity has been determined, age reading is rather simple.

Otolith rings in tropical species are rather less clearly defined than in cold-water fish: the predominance of transparent zones requires methods which can bring out the contrast between the opaque and the hyaline rings. For interpretation, the otoliths can be immersed in a dense clarifying liquid (clove oil, liquid paraffin, glycerine) in a dark container. Reflected light and a binocular microscope are used for the reading. Slow growth rings will appear dark through the dark transparent

background of the container, whereas fast or opaque growth rings will appear light under reflected light. Move the light focus and alter the positioning of the otoliths to help differentiate the growth rings: avoid fixed mounting media which will not let you touch the otoliths.

Thick, opaque otoliths can be interpreted after they have been left for some time in a clarifying liquid such as water or glycerine. The otoliths can be positioned in numbered ice-cube trays containing the liquid selected and left to clarify. The time depends on the species but 12-24 hours is enough for most. Another technique is to heighten the contrast between the growth rings by burning the otoliths (Christensen, 1964). The slow, hyaline growth rings which contain more protein (Casselman, 1974) acquire a darker, caramelized tone when burned. The otolith is burned on a metal sheet under a low flame (Bunsen burner) or in an oven at 100°C. Burning time depends on otolith size and flame heat. The process must be carefully monitored to avoid burning the otolith and thereby losing it. The otoliths may be stained with a protein-affinity dye.

If necessary, otoliths can be polished or sliced to read the macrostructural growth pattern (Fig. 8). Generally speaking, fish which reach a great age and grow slowly can be aged more precisely in sections than by reading the whole otolith. Reading whole otoliths for young specimens and sections for larger fish can facilitate the study and produce good results.

Ring periodicity can be read by following the progression of the rings formed on the edge of the otolith throughout the year. A graph of the monthly percentage of otoliths with opaque and hyaline borders will show the ring deposition period. In the case of annual formation, one maximum per year will be found for each type of ring (Fig. 9).

Once the daily periodicity of the increments has been determined for a species, the number of annulae increment components can be used to determine how frequently these growth structures form. When the number of increments in one opaque ring and one hyaline ring does not differ significantly from 365, annulae formation can be considered to be annual. This method is applicable even when daily increment periodicity has not been determined to obtain an approximation of annulae periodicity. In this case, however, the results obtained must be validated by other methods.

Fig. 8. Sagitta of Merluccius capensis showing seasonal growth rings. The slow (hyaline) growth rings appear darker (arrows) under incidental lighting. In the upper part of the figure there is a cross-section of the otolith. Note the numerous false (non-seasonal) rings which form in this species.

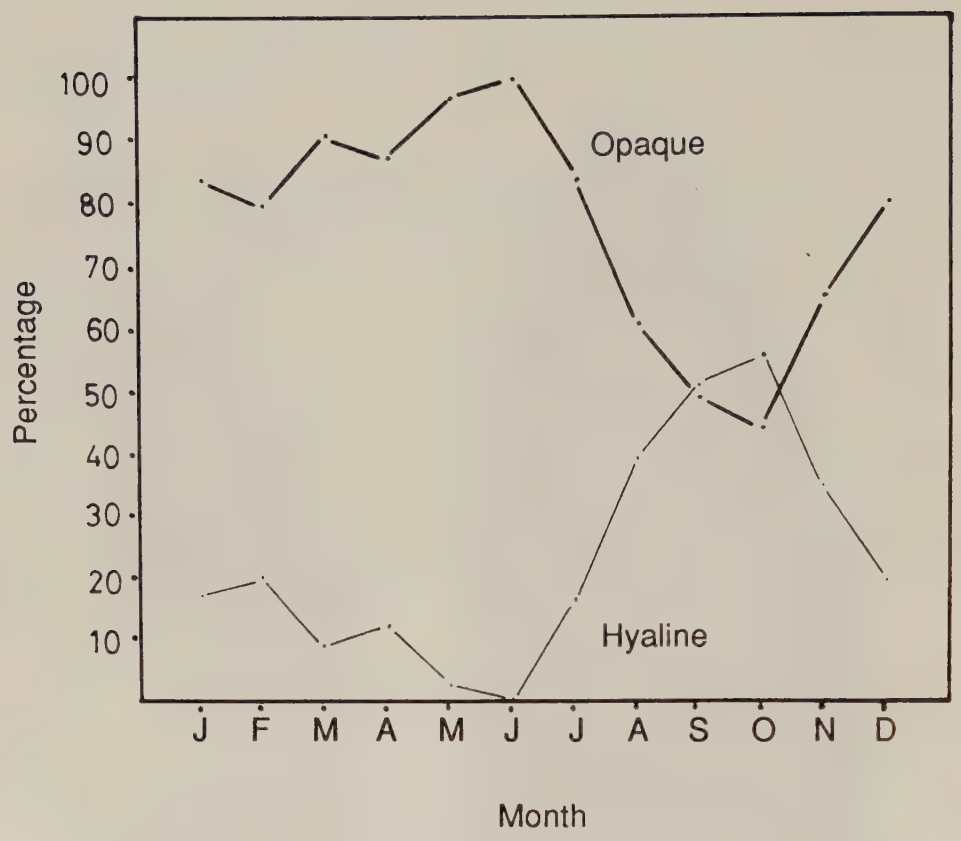

Fig. 9. Yearly variation of the percentage of otoliths with a rapid (opaque) growth ring on the edge.

After measuring the distance from the nucleus to each ring, ring distances can be plotted for each age group. This will give a unimodal distribution in approximately the same places for each of the growth rings (Fig. 10) when ring formation is regular and corresponds to a seasonal growth pattern common to the stock (Manooch III, 1987; Taubert and Coble, 1977).

After counting the rings and determining their annual periodicity, the age has been established. A fish's age is the interval between birth up to a given point in time, usually capture. The age class or annual group is determined from the birthday. The birthday of a given fish is unknown and therefore arbitrary birthdays are used for an entire stock based on maximum spawning or other decisive factors in recruitment. The standard birthday in the northern hemisphere is 1 January and in the southern 1 July. Other dates can be used as convenient, however.

6. VALIDATION TECHNIQUES

Age structure determination of a fish stock from growth structures is based on the assumption that growth increments occur simultaneously at standard intervals within a specific cohort, in the form of regular depositions of new growth structures. In beginning the study of a stock, compliance with the two basic criteria: periodicity and synchronous development, must be assured. Extrapolation of the results from other populations is not a valid technique (Beamish and MacFarland, 1983).

Validation methods can be divided into direct and indirect methods. The lapse of time between two determined events is known in direct validation and therefore the periodicity of the increments can be read. In indirect validation methods, the medium lengths of each age class, read on the basis of growth increments, are compared with those obtained by other methods. When the results concur, the use of growth increments as a technique is considered valid. This approach is used with species that cannot be reared or maintained in captivity and with those for which only fishery data are available.

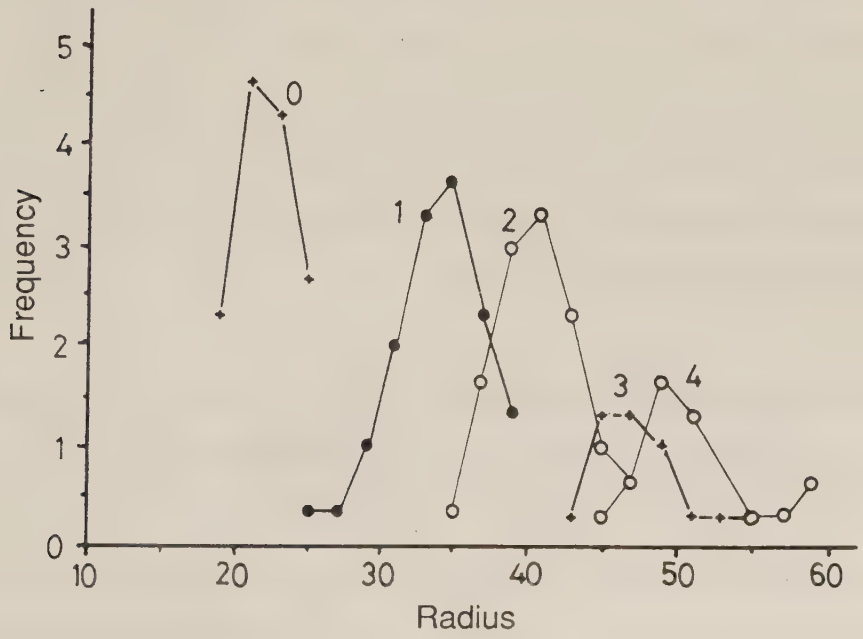

Fig. 10. Distribution of distances from the centre of the otolith to each ring considered to be an annual ring in Lethrinus.

6.1 Direct methods

a) Rearing and sequential sacrifice

The most precise method of determining increment periodicity is to rear the fish and to sacrifice them in sequence. In examining the otoliths, the age at which ring formation begins will be determined (number of days between spawning and first increment) as will increment periodicity.

The interval between spawning and reabsorption of the vitelline sac requires intensive study to determine the exact start of formation. The start depends on the length of the embryonic period and the fish species. Generally speaking, the beginning coincides with spawning or with the onset of active feeding (Brothers et al., 1976; Geffen, 1983; Marshall and Parker, 1982; Mcgurk, 1984; Neilson and Geen, 1982; Stenffensen, 1980; Wilson and Larking, 1980; Yzeng and Yu, 1988).

In plotting the number of otolith increments against the number of days from birth, you will obtain a lineal slope relationship not significantly distinct from I. The ordinate in the abscissa axis will indicate age at the onset of deposition. The number of samples to be observed is determined by assigning a confidence limit of 95 percent in calculating individual age from the linear relationship.

b) Marking otoliths

Otoliths can be marked or tagged with a systemic chemical which acts as a reference, thus making it possible to work with fish of unknown age, as the interval between tagging and sacrifice is known, making it easy to determine growth (Wild and Foreman, 1980).

Capture, tagging and release into the environment has been done with hardy species which survive the resultant stress and which can be tagged in sufficient numbers to guarantee recapture. Although their growth will be affected by handling, it will also reflect growth under natural conditions.

When fish are held in captivity, a period of acclimatization must be allowed before beginning the experiment so as to avoid stress-caused growth slowdowns and the consequent checks or discontinuities in the otolith.

Tetracycline is the most common marker: it acts to chelate the calcium and magnesium present in the calcified tissues. Under ultraviolet light and the fluorescent microscope, the tetracycline band will appear as a yellow ring against the green background of the otolith. Tetracycline is broken down by light and so the otoliths must be kept in the dark (Chilton and Beamish, 1982).

Tetracycline in the form of OTC (tetracycline hydrochloride) is offered in the fish feed or given as an intraperitoneal injection at concentrations of 25-30 mg per kilo of fish weight (Wild and Foreman, 1980). Mass marking of larval and juvenile fish involves their immersion for 120 minutes in a 500 mg/l solution of tetracycline in distilled water with 3.5 percent of buffered NaCl at a pH of 6.0-6.2 (Hettler, 1984; Schmitt, 1984).

A large number of specimens in marking experiments often present diffuse fluorescent bands or none at all (Chilton and Beamish, 1982). In marking experiments with Lutjanus kasmira, evidence emerged to show that the amount of tetracycline fixed during periods of slow growth was too small to produce a readable fluorescent band (Morales-Nin, Ralston and Uchiyama, unpublished data).

Otoliths can be marked for SEM viewing, producing visible growth checks induced by immersing the otoliths for 18 hours in an acetolamide solution of 125 ppm (Ralston and Miyamoto, 1981) or through experimental stress induced by anoxia or thermal shock (Fig. 11) (Pannella, 1980).

It is common in this type of experiment to encounter broad individual variation in the time and frequency of formation of the mark in the otolith. Therefore, many individuals must be marked to guarantee that the experiment is productive. Fish which are not clearly marked or which have a diffuse fluorescent ring are very frequent.

The ratio of days after marking to the increments formed will indicate the process of formation. A linear ratio starting at 0 indicates correspondence (number of days = number of increments): when the origin is other than zero it indicates a period of latency prior to the onset of formation. Where the relationship is non-linear, there may be no correspondence, or the increments may be too fine to be detected under the light microscope.

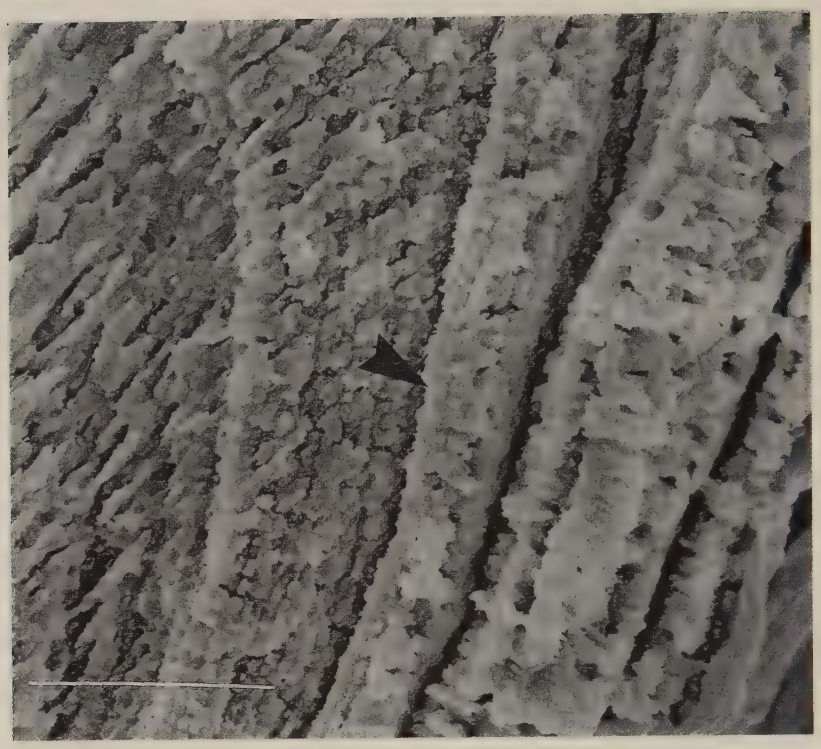

Fig. 11. Otolith growth check caused by severe environmental stress. Scale 12 μm.

6.2 Indirect methods

a) Assigning a birthday

The birthday of an individual fish can be back-calculated from the date of capture and the age reading (Methot, 1983). A comparison of this date with the spawning period of the species will provide a check on the precision of the age reading.

The method is only applicable to larval and juvenile fish as selective mortality can alter the results in adults. Discrepancies between the estimated date of birth and the spawning period may be due to growth checks or deviations in daily periodicity (Geffen and Nash, 1985).

b) Growth patterns in otoliths

The cyclical growth patterns - weekly, monthly and seasonal which form in otoliths (Fig. 12) depend on regularly occurring external factors. When patterns of growth are regular and attributable to an environmental stimulus, their periodicity allows the determination of increment formation frequency. In coastal species with cycles

of 7-14-28 increments, daily periodicity in the otoliths may be assumed (Geffen, 1987). Similarly, the number of increments in a seasonal ring has been used to determine periodicity (Gjosaeter et al., 1983).

The metamorphic phases in the larval period are reflected in the otolith's nuclear structure (Brothers and MacFarlan, 1981; Marshall and Parker, 1982). The age of the various metamorphic phases is known for many species and can therefore be related to the otolith structure (Geffen, 1987).

c) Synchronous increment formation

Increment deposition depends on an internal rhythm which is synchronized with environmental fluctuations and prompted by a cyclical stimulus (Campana and Neilson, 1985). A group of fish subjected to the same environmental conditions should form their increments at the same time. By repeatedly sampling the fish throughout a day-period, the development of the increment on the edge of the otolith can be followed and its periodicity determined.

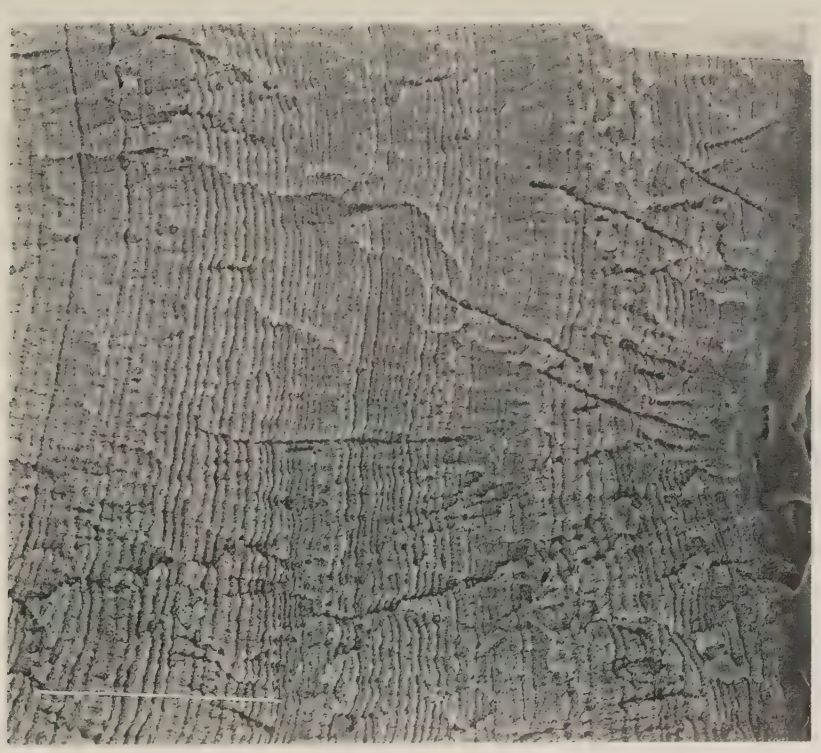

Fig. 12. Cyclical groups: otolith growth in Haplochcomis, probably related to rhythmic environmental variations (scale 86 μm).

The percentage of marginal increment deposition (c), determined by measuring the increment in the process of formation (In) and of the one immediately anterior to it (In-I):

$$c = 100(In/In-I)$$

will make it possible to determine the time when the increment was formed (Mugiya et al., 1981).

This method has been applied to fish reared in captivity (Morales-Nin, 1985 b) and to fish who remain in the same area and do not migrate vertically during the day (Ré, 1983). The method is only applicable to larval and juvenile fish, as the marginal increment of adult fish otoliths is usually damaged during preparation.

d) Progression of the median age, if the population is made up primarily of one age class which, once recruited, does not migrate. In this case the evolution over time of the mean ages will make it possible to determine the periodicity of increment formation (Darayatne and Gjosaeter, 1986).

e) Comparison of average lengths

Similarity between growth curves plotted from increments and those plotted from other methods can provide validation of the age readings determined by counting increments. In comparing the various growth curves, similar length ranges should be considered as the rate of growth varies with age.

7 ESTIMATING GROWTH BY LENGTH-FREQUENCY ANALYSIS

The length distribution in a sample from a specific population is the product of recruitment, growth, mortality and sampling errors. Annual variations in recruitment and individual variability in growth frequently mask the interpretation of modal classes in length frequency.

These methods are based on the assumption that each modal class in a frequency distribution will correspond to a cohort and represent different age classes determined at regular intervals. The presence of modes in the length distribution depends on the distance between the medians, the extent of the variance, the proportion of each age class in the population and the size of the sample studied (MacDonald and Pitcher, 1979; Fournier, 1983).

Methods of growth determination based on length-frequency analysis can be applied to populations with a markedly seasonal recruitment, where the identity of the year classes is maintained, and when growth is sufficiently swift to avoid excessive superimposition of the lengths of successive age classes.

Gear selectivity can influence the type of length frequency. The smaller lengths, for instance, not fully recruited to the gear, will be under-represented. Selectivity can be corrected by calculating the capture probability (Brey and Pauly, 1986). When the gear is also selective with respect to larger-sized fish, as with trammels and long-lines (Ralston, 1990), it is much harder and sometimes impossible to use methods based on length frequency analysis.

Hosmer (1973) showed that the estimation of the parameters of each age class, such as average length and relative abundance, are enormously facilitated by having separate samples from one or more age class. This author formulated the calculations needed to identify two normal components in the frequency distribution. Based on these original calculations, more general systems were developed for a larger number of components.

A time sequence of length frequency distributions makes it possible to separate age classes which might otherwise be obscured by the super-imposition of frequencies. Changes over time in each cohort can be analysed visually (Petersen, 1891), or by computerized methods developed from Hasselblad (1966), which separate the modal classes of the length-frequency (MacDonald and Pitcher, 1979; Schnute and Fournier, 1980). Pauly and David (1981) analysed the time series, assuming that the mode of each class followed a von Bertalanffy curve (1938; 1957; 1964).

Various approaches have been utilized in the process of selecting modes in a single frequency; graphic methods which determine the area of the cumulative frequency (Cassie, 1954; Bhattacharya, 1967) and statistical methods based on maximum likelihood. Growth parameters in the second group can be determined by adapting complex models to length-frequency (Schnute and Fournier, 1980). These hypotheses can be determined and verified when specific characteristics are attributed to the processes (e.g. normal length distribution in each age class) and likelihood functions are maximized.

Assumed growth models can be adjusted by a minimum chi-squared method or other technique to the modal classes observed in the frequency (Pauly, 1984; Pauly and Gaschutz, 1979; Pauly and Morgan, 1985). Recently, Wetherall et al., (1987) developed an ingenious method for calculating Loo and Z/K based on scant length-frequency data.

7.1 Joint application of age data and length-frequency

The determination of median lengths and relative abundance in each cohort is more precise when a subsample of age data is available for one or more of the age classes present in the length-frequency (Hosmer, 1973). Given the close correlation of the von Bertalanffy parameters, Loo and K, errors in plotting from the same set of length-frequency data will be avoided by including in the calculation data which is not dependent on growth. Age determination of a length-frequency subsample makes it possible to know the number of age classes in the population and make the calculations more accurate.

MacDonald and Pitcher (1979) stated that the use of age data limits the number of possible components in length distribution and implies adjustments of greater biological significance. Comparably, Morgan (1987) applied age data to improve growth parameters calculated from length-frequency. A modification of Morgan's method was developed by Gayanilo et al (1988).

The necessary age subsample for the application of the above methods can be selected at random from the length-distribution, or by stratified sampling. The catch of most species is made up of various age classes of different abundances, and therefore the size-stratified sample will eliminate the errors introduced by the relative abundance of lengths and will allow more of the bigger fish to be sampled where the superimposition of lengths is greater.

8 OTHER GROWTH DETERMINATION METHODS

8.1 In vitro growth

Ottaway and Simkiss (1979) and Ottaway (1978) developed a method of incubating scales in vitro with C^{14} marked glycine. The scales must be extracted from live fish and incubated immediately with the glycine at temperatures like those in their habitat. The amount of radioactive glycine fixed by the osteoblasts which remain stuck to the scales by the end of a given period provides a basis for determining the rate of growth.

In normal cells, each cell has a fixed quantity of DNA, regardless of individual physiological condition. NRA present in the cell, on the other hand, is a direct anabolic function. The proportion of DNA and RNA is therefore an indicator of protein synthesis and growth (Bulow, 1987).

8.2 Radiometry

This method is based on the radioactive imbalance of the Ra^{226} fixed during otolith formation. The radioactive decay of this element produces Pb^{210}. The proportion of the two isotopes indicates how much time has gone by since the incorporation of the Ra^{226}. The Pb^{210} isotope is appropriate for age determination as its half-life of 22.3 years is comparable to fish longevity (Bennet et al., 1982; Campana et al., 1990).

The method requires species with a long life-span like Sebastes which can reach the age of 80, in which the changes can be evaluated (Bennett et al., 1982).

8.3 Lipofucsin analysis

Lipofucsin is a lipopigment which forms increasingly with age in the cytoplasm, and is considered one of the clearest indicators of ageing in post-mytotic cells (Nandy, 1985).

The lipofucsin build-up in krill (Euphasia superba) has been used to determine the age composition of the population (Ettershank, 1985). Its presence in cerebral tissues is a function of age and can be used to determine fish age (Hill and Radtke, 1988; Hill and Womersley, 1991).

8.4 Marking and recapture

Growth can be measured directly by capture, marking and subsequent release into the environment (Ikenouye and Masuzawa, 1968). Where a set of initial length values and length at recapture are available, and the time between tagging and recapture is known, then growth can be determined by the method of Gulland and Holt (1959), Ford-Waldford (Ford, 1933) and Munro (1982).

8.5 Direct observation of growth

Growth can be observed directly in fish raised in captivity, where length progression can be followed throughout the rearing period. Growth in captivity is not, however, comparable to growth under natural circumstances.

Growth can be established in sedentary populations which can be observed in the same place over long periods of time. This information is obtained by photographs or by drugging the fish and measuring them directly.

The sets of data obtained should make it possible to use the Gulland and Holt method (1959), or other methods (see 8.4).

9 CLOSING REMARKS

Fish age and growth can be determined by various methods which have an element of subjectivity and therefore require knowledge of the fish species in order to select the option which makes the most sense, biologically speaking.

Age determination from seasonal rings is feasible in most species. The method offers information on length variability in each year class, making it possible to establish whether length-distribution at each age is normal. Normal distribution is an assumption basic to many length-frequency analysis methods (Morgan, 1988). Although the otoliths of many tropical fish species have seasonal rings, ring formation is not universal. Frequently, in the same area, some species have rings with seasonal periodicity and others do not (Morgan, 1987).

Slow growth rings are deposited following periods of environmental or physiological stress such as spawning, migration or environmental fluctuations. In some species a link has been established between ring formation and the period of sexual maturity. The reproductive process involves striking changes in feeding and metabolism which are mirrored in bodily growth. Although sexual cycles may be one of the most important causes in ring formation, ring formation in immature fish implies some other triggering factor. A deeper understanding is needed of fish metabolism and physiology if the mechanisms of otolith deposition are to be established.

The rings in tropical fish are usually rather indefinite, and the interpretation of the pattern often overshadowed by the presence of false and multiple rings. Methods such as otolith burning, which enhance the contrast of the real, and (normally) structurally different rings, therefore need to be used.

The consistency of an interpretation must be tested by the available statistical techniques (Beamish and Fournier, 1981). In otolith interpretation, neither the size of the specimen nor other factors which might influence the result must be known.

Once the presence of rings which can consistently be interpreted has been established, the temporal significance of their deposition must also be established. The type of sample available will determine the applicable age validation method. The most useful method is probably to follow the evolution of rings on the edge of the otolith, in that it allows the time of formation and possible causes to be established.

Increment depositions in adult fish during slow growth periods may be very fine (0.1-0.4 μm) which are undetectable with the light microscope (approximate amplification 1 μm), and will appear to the reader as bands with no clear increments. The resolution may cause rhythmic increments to be perceived as a single increment and lead to underestimations of age.

The presence of these fine increments is a serious limitation to the application of light microscopy in determining the age of adult fish. In starting the study of a species it is advisable to read with the SEM some otoliths which cover the length range and thus to determine whether there are fine increments which would rule out the use of the light microscope. The cost of the scanning electron microscope and the time needed to prepare the otoliths and view them, however, make the application of this method impracticable for the numerous otoliths which must be interpreted in a fishery consultancy. In species with minimal growth periods, use the light microscope to determine age in the early growth phases and the scanning electron microscope to determine the rate of growth in adults and to validate findings obtained by other methods.

The first phase of study should determine the best method of otolith preparation, the presence and clarity of the growth rings, and the criteria for interpretation. When growth rings are found, their periodicity must be established before going on to determine the age.

When you wish to use growth increments, you must study other adult fish otoliths with the SEM to determine whether light microscopy is applicable. A combination of electron and light microscopy is the most cost-effective and time-saving approach.

10 GLOSSARY

Glossary of terms used (Jensen (1965), with modifications).

Term - (Spanish equivalent) - synonyms - definition

(A) Amstrong - A measure used in microscopy equal to the 10 millionth part of 1 mm.

Age - (edad) - Period since birthday (in days, months or years).

Annual class - (clase anual) - Group of fish born the same year. The year class is based on an arbitrary birthday according to maximum spawning period, international agreements, or the capture date.

Annual ring - (anillo anual) - annulus - Seasonal ring: Annually formed growth structures.

Annulus - Fixed-periodicity concentric ring allowing age determination. These rings appear opaque or hyaline (transparent) depending on optical refraction and generally attributed to fast and slow growth periods, respectively.

Bands - (bandas) - Rings, zones, areas: Auxiliary terms used to describe growth structures.

Check - (discontinuidad) - An abrupt discontinuity in an otolith ring or structure.

Cohort (cohorte) - Group of fish born during the same spawning period and beginning life together.

Crystalline otoliths - (otolitos cristalinos) - Fully or partially anomalous otoliths, made up of calcite and which cannot be used for age determination.

Focus - (foco) - origin: the hypothetical or real point on the otolith where the count begins, or which is used as the source of the measurements.

Focus - (nucleolo) - The area or areas with no growth structures around the primordial otolith.

Hyaline ring - (anillo hialino) - Slow growth ring: ring through which light can pass.

Increment - (incremento) - Daily growth increment, daily ring: a growth structure formed daily made up of two zones in which calcium and organic matter predominate; respectively.

μm - Micra - (micrómetro) - A measure used in microscopy equivalent to one thousandth of a millimetre (1 mm = 1 000, μm = 10 000 000 A).

Marginal increment - (incremento marginal) - Edge, border: the area laid down just after the last identifiable growth structure at the edge of the otolith.

Nucleus - (nucleo) - Otolith zone formed just before the first ring.

Opaque ring, fast growth ring - (anillo opaco) - Fast growth ring: a ring too dense to allow the passage of light.

Primordium - (primordio) - Zone where otolith growth begins. Appears optically as a more opaque area within the nucleus.

Radius - (radio) - The linear distance from the focus to a specific point on the edge of the otolith.

Validation, age - (validación) - Confirmation of the temporal significance of a ring or increment.

Verification - (verificación) - Confirmation of the repeatability of a numerical interpretation of age (may be independent of actual age).

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